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ORIGINAL PAPER

Plasticity to wind is modular and genetically variable in *Arabidopsis thaliana*

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Abstract Thigmomorphogenesis, the characteristic phenotypic changes by which plants react to mechanical stress, is a widespread and probably adaptive type of phenotypic plasticity. However, little is known about its genetic basis and population variation. Here, we examine genetic variation for thigmomorphogenesis within and among natural populations of the model system *Arabidopsis thaliana*. Offspring from 17 field-collected European populations was subjected to three levels of mechanical stress exerted by wind. Overall, plants were remarkably tolerant to mechanical stress. Even high wind speed did not significantly alter the correlation structure among phenotypic traits. However, wind significantly affected plant growth and phenology, and there was genetic variation for some aspects of plasticity to wind among *A. thaliana* populations. Our most interesting finding was that phenotypic traits were organized into three distinct and to a large degree statistically independent covariance modules associated with plant size, phenology, and growth form, respectively. These phenotypic modules differed in their responsiveness to wind, in the degree of genetic variability for plasticity, and in the extent to which plasticity affected fitness. It is likely, therefore, that thigmomorphogenesis in this species evolves quasi-independently in different phenotypic modules.

Keywords Genetic differentiation · Mechanical stimulation · Phenotypic integration · Phenotypic plasticity · Thigmomorphogenesis

Introduction

In the life of a plant, mechanical stress, through direct contact with insects and neighbouring plants or flexure caused by wind, water or snow, is a frequent phenomenon. Plants

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respond to these mechanical stimuli in a variety of ways (Biddington 1986; Braam 2005). Some of these are very rapid, like the well-known responses of stinging nettles, mimosas and carnivorous plants; others are slow and involve complex adjustments of plant form and function during ontogeny. A familiar example of the latter is the extremely compact growth of trees near the tree line in many mountainous areas, the so-called Krummholz phenomenon. Slow, developmental responses to mechanical stimulation have been termed thigmomorphogenesis (Jaffe 1973), and research has shown that mechanical stimulation causes, among others, decreased elongation, increased stem thickness and tissue strength in many plants (reviewed in Biddington 1986; Jaffe et al. 2002; Braam 2005). Because of its potential relevance for horticulture and forestry (Ennos 1997; Latimer 1998), much of the experimental work on thigmomorphogenesis has been done in crops (e.g., Biddington and Dearman 1985; Garner and Björkman 1996) and trees (e.g., Lundqvist and Valinger 1996; Kern et al. 2005).

Another avenue of research has been the study of the molecular basis of thigmomorphogenesis in the model system *Arabidopsis thaliana*, which led to the discovery of a set of candidate genes—the TCH gene family (Braam and Davis 1990)—and begun to unravel the complex mechanisms controlling TCH gene expression and the functions of TCH gene products (e.g., Sistrunk et al. 1994; Xu et al. 1995; Braam et al. 1996; Johnson et al. 1998). A recent debate on whether visitation by scientists can affect the outcome of field experiments (Cahill et al. 2001; Malakoff 2004) has even made plant ecologists familiar with the idea that inadvertent mechanical stimulation can have profound effects on plants.

In spite of all this progress, we know virtually nothing about the evolutionary biology of thigmomorphogenesis. Arguably, a thorough understanding requires to go beyond the description of phenotypic patterns and physiological mechanisms, and to ask questions about the origin, potential adaptive value, and evolutionary dynamics of thigmomorphogenesis.

From an evolutionary ecological point of view, thigmomorphogenesis is developmental phenotypic plasticity in response to mechanical stimulation. If this ability to respond to environmental variation affects plant fitness, at least under some conditions, and it has a genetic basis, then it will be (or has been) subject to natural selection, and it is likely to have evolved as an adaptation for plants to cope with wind and other forms of mechanical stress (Jaffe et al. 2002; Pigliucci 2002; Braam 2005). A rigorous demonstration of the adaptive nature of thigmomorphogenesis, however, is not a trivial task and requires the use of several tools from the conceptual and empirical arsenal of evolutionary biology, including: (1) common garden studies that test for genetic variation in thigmomorphogenesis, i.e. its potential to evolve; (2) selection studies that demonstrate a fitness advantage of thigmomorphogenesis under natural conditions; and (3) comparative studies that explore the evolution of thigmomorphogenesis in a broader phylogenetic context. While these methods have been successfully applied to the study of other plasticity syndromes, in particular shade avoidance (e.g., Schmitt et al. 1999; Pigliucci et al. 1999; Callahan and Pigliucci 2002), they have hardly been used to study thigmomorphogenesis.

Here, we present a comprehensive study of the genetic variation for thigmomorphogenesis among and within natural populations of *A. thaliana* (L.) Heyhn. It is the first in a series of papers that will examine the ecological and evolutionary genetics of thigmomorphogenesis in this model system. Because of the previous work on candidate genes for thigmomorphogenesis, and because we have some evidence of genetic variation among natural populations (Pigliucci 2002), *A. thaliana* should prove to be an ideal system for integrating ecological and molecular approaches to thigmomorphogenesis.

In a preliminary study, Pigliucci (2002) found significant genetic variation for thigmomorphogenesis among 11 natural accessions of *A. thaliana*. Moreover, his results suggested that mechanical stress exerted by wind alters phenotypic correlations in *A. thaliana*, and that there might be a general difference between early- and late-flowering populations in this respect. The purpose of the present study was to build on these findings by using several maternal families from each of 17 natural populations that we collected across a broad geographic gradient in Europe, where *A. thaliana* is native. Specifically, we addressed the following questions: (1) How does mechanical stress through wind affect the phenology, growth form and fitness of *A. thaliana*? (2) Is there genetic variation for thigmomorphogenesis among natural populations of *A. thaliana*, and if so, how is it distributed among families, populations, and geographic regions? (3) Is there evidence for a plasticity "syndrome", i.e. a consistent and significant effect of mechanical stimulation on patterns of phenotypic integration in *A. thaliana*? (4) Is there a dichotomy between early- and late-flowering ecotypes with regard to their plasticity to wind, as hinted at by previous research? (5) What is the relationship between the degree of this plasticity and plant fitness?

Materials and methods

Study species

Arabidopsis thaliana is a small annual weed in the mustard family (Brassicaceae). It is a predominantly selfing species native to Eurasia but now widely naturalized in the USA and elsewhere. *A. thaliana* typically occurs in open or disturbed habitats such as agricultural fields, roadsides, river banks, rocky slopes, sand dunes, sparse meadows or waste places (Mitchell-Olds 2001; Al-Shehbaz and O'Kane 2002) where mechanical stress through wind should be a frequent phenomenon.

Initially, *A. thaliana* has been a model species for plant genetics and molecular biology (Pang and Meyerowitz 1987; Meinke et al. 1998). However, the growing body of knowledge about its development and molecular biology, coupled with the logistic advantages it affords and the availability of a broad array of molecular tools, have also made it a popular model species for addressing basic questions in ecology and evolutionary biology (Mitchell-Olds 2001; Koornneef et al. 2004; Tonsor et al. 2005). Broad-scale surveys of genetic variation in natural *A. thaliana* populations usually find substantial variation among populations, and sometimes also within populations (e.g., Bergelson et al. 1998; Jørgensen and Mauricio 2004; Nordborg et al. 2005; Stenøien et al. 2005). While there is often little geographic structuring in the distribution of neutral molecular variation (but see Sharbel et al. 2000), genetic variation in quantitative traits is often non-random in *A. thaliana*. For instance, variation in phenological traits correlates with latitude (e.g. Stinchcombe et al. 2004), and Stenøien et al. (2005) found Q_{ST} values to be significantly lower than F_{ST} values in Northern European populations of *A. thaliana*, suggesting stabilizing selection on quantitative traits. Taken together, natural populations of *A. thaliana* seem to be characterized by both frequent gene flow and rapid local differentiation.

In this study we used plant material from 17 natural populations of *A. thaliana* that were sampled across a broad geographic gradient in Europe. In the summer of 2001, mature fruits from several mother plants were collected in four natural populations around Barcelona (Spain), five populations around Orsay and one outside Dijon (both France), six populations around Wageningen (Netherlands), and two populations outside Uppsala

(Sweden). To reduce the possibility of maternal environmental effects, all maternal lines were propagated in a common environment for at least two generations prior to the experiment.

Experimental design

In October 2004, seeds from three maternal families per population were placed on wet filter paper and cold stratified for 1 week at 5°C. Germinated seeds were transferred to planting trays filled with a standard potting soil (Pro-Mix BX, Premiere Horticulture, Dorval, Canada). The plants were placed in an unheated greenhouse and eventually thinned down to one seedling per cell. After 2 weeks, one seedling per maternal family was transplanted into each of 24 96-cell trays filled with the same soil as above. Because of its low germination success, one maternal family from a Swedish population was excluded, so the eventual size of the experiment was 50 families times 24 trays = 1,200 plants. In each tray, the locations of seedlings were assigned randomly. In December 2004, when all plants had well established rosettes, the trays were transferred to a growth chamber where they were kept at 5°C for another 4 weeks. Since there is genetic variation for vernalization requirements in many natural populations of *A. thaliana*—some genotypes require a vernalization period to flower, others do not (Lempe et al. 2005)—we used the growth chamber to make sure all plants were phenologically synchronized and ready to flower. After that, we brought the plants back to the greenhouse and started the mechanical stimulation treatments.

Mechanical stress in our experiment was created by subjecting plants to simulated wind. In Central Europe, the mean wind speed at 50 m above ground is 5–10 m/s, depending on wind exposure (Troen and Petersen 1989). With a logarithmic profile of wind speeds (Oke 1987) this corresponds to an upper limit of approximately 2–3 m/s at a height of a few decimetres, which would be relevant to a small plant like *A. thaliana*. We used high velocity fans (Patton PX400-UC; The Holmes Group, Fontana, CA) to create three levels of wind intensity: control, low wind, and strong wind. In the low wind treatment, the average wind speed, measured with an anemometer, was approximately 2 m/s. Based on the wind statistics mentioned above, we considered this to be a frequently experienced, "normal" amount of wind and mechanical stimulation. In this treatment, the plants were significantly bent and shaken, but the stress was not extreme. In the strong wind treatment, the average wind speed was approximately 5 m/s. Here, the plants were strongly bent and thrown around, and the overall mechanical stress appeared to be rather intense.

The experiment had a block design with four spatial blocks in an unheated greenhouse bay, three wind treatments per block, and two replicates per seed family (= two planting trays) in each treatment. Within each block, the locations of wind treatments were assigned randomly. The wind treatments were applied from 6–9 AM and 6–9 PM each day, i.e. for a total duration of six hours per day. Since the fans produced a highly directed air stream, there was very little interference with nearby treatments and the wind speed in the control treatment, even when adjacent to strong wind, was close to zero, as measured by an anemometer. The plants were bottom-watered frequently throughout the experiment. No additional lighting was used, i.e. the plants were exposed to normal ambient light. To ensure even exposure to wind, all trays were rotated weekly. The total duration of the experimental treatments was 2–3 months, depending on the individual phenology of a family. The plants were subjected to the wind treatments during the bolting, flowering, and fruit ripening periods. There was very little indication that wind affected plants through

direct damage such as stem rupture or uprooting. Only 2–3 times did we observe a side branch that appeared to be broken due to wind action.

To characterize the phenotypic response of *A. thaliana* to mechanical stress, we measured a total of nine variables. For each of the 1,200 plants we recorded: (1) the time to flowering (first white of petal visible), (2) the leaf number at flowering, and (3) the rosette diameter at flowering. After that, one experimental block of 300 plants was used to take samples for a gene expression study (J. Braam et al. in prep.), so the remaining traits were measured on 900 plants. In each of the remaining plants we measured (4) the length of the reproductive period (first flowering to first fruit dehiscence). Two weeks later we harvested the plants, dried them at 80°C for at least 24 h, and measured (5) plant height, (6) aboveground biomass, and (7) the total number of fruits produced. In addition, we measured two architectural traits that were expected to be informative in this context (Cipollini 1999; Pigliucci 2002): (8) stem diameter (2 cm above the base) and (9) the total number of basal and lateral branches, as a description of overall growth form.

Statistical analyses

To improve normality of the data and homogeneity of variances prior to parametric analyses, all variables were Box-Cox transformed. As a first inspection of the data, we ran a principal components analysis on the correlations between transformed variables across treatments. Next, each variable was analyzed separately with nested analysis of variance using type III sum of squares. The standard ANOVA model included block, treatment, and their interaction, as well as the nested effects of region, population within region, seed family within population, and the interactions of these factors with the treatment. The main effects of block and treatment were tested against the residual mean squares. We did not use the block by treatment interaction as error term, as suggested by some authors (see Newman et al. 1997), because in our experiment the blocks were best thought of as a fixed effect set up to systematically capture the environmental heterogeneity of the greenhouse space. Region effects were generally tested against the respective population effects, and population effects were tested using the mean squares of the respective family effects as error terms. Because this is a rather conservative way of testing a nested model, we generally also considered region and population effects that were marginally significant ($0.1 < P < 0.05$). To get a better idea of effect sizes and the relative importance of each genetic and environmental effect in the ANOVA, we also calculated the % variance explained (SS_{effect}/SS_{total}) by each model effect and interaction.

Because there was a pronounced bimodal distribution of flowering times, and we were interested in the possible role of flowering "ecotype" in touch response, we classified plants as either early- (<106 days) or late-flowering and included flowering type and its treatment interaction in a separate set of ANOVAs.

Moreover, we investigated whether the correlation structure among phenotypic traits was altered by the wind treatments, i.e. if there was a multivariate plasticity syndrome, by calculating separate phenotypic correlation matrices for each treatment and testing for their pairwise similarity with Mantel tests and, as there is no consensus on the best method for matrix comparison (Steppan et al. 2002), also common principal components analysis.

Finally, we tested for selection on plasticity to wind by examining family-level genetic correlations between plasticity and estimated average plant fitness across wind environments (Scheiner 1993; Via et al. 1995). Plasticity in response to wind was estimated in two different ways: (1) as the coefficient of variation (CV) of a trait across all treatments, or (2) as the percentage change of the trait mean from the control to the strong wind treatment

(which is equivalent to the slope of the reaction norm across these two treatments). Selection on plasticity was estimated by calculating the correlations between these plasticity estimates and the average fitness of a family across environments.

However, in the case of a simple fitness average, all environments are given the same weight, which assumes they are equally important in nature. This may not be a realistic assumption, and we therefore explored the sensitivity of plasticity-fitness correlations to different frequencies of wind environments. In particular, as *A. thaliana* commonly occurs in open habitats, it probably rarely encounters windless environments. We therefore calculated additional fitness averages in which either the low wind or the strong wind, or both, were given a threefold weight, compared to the control treatment. We recalculated the plasticity-fitness correlations with these modified fitness averages.

In addition, we tested for possible costs of plasticity (DeWitt et al. 1998) by correlating the same plasticity indices as above with plant fitness in the control treatment.

Results

Principal components analysis indicated that there were three major axes of variation in our data (Table 1). The first axis was mostly associated with traits (directly or allometrically) related to plant size: plant height, biomass, and fruit number, as well as stem thickness and diameter at flowering. The second axis was strongly associated with flowering time, leaves at flowering, and length of the reproductive period, with the latter negatively correlated with the other two. Both of these axes explained about one third of the total variation in the data. Finally, a third axis was almost entirely associated with variation in the total number of basal and lateral branches. Broadly speaking, these three axes may be taken to represent phenotypic variation in size-related traits, in phenology, and in growth form, respectively. Since the traits constituting each axis are at least partly independent from each other, so might be their response to the wind treatments, and we therefore examine in the following the traits in separate groups, as identified by the multivariate axes of variation.

There was a significant treatment main effect on all of the traits associated with the plant size axis (Table 2). However, only in three traits the wind treatments explained a significant percentage of the total variance (Tables 3 and 4): on average, plants in the strong

Table 1 Results of a principal components analysis of all phenotypic traits pooled across wind treatments. For each trait the highest factor loading is indicated in bold

(% variation explained)	PC 1 "Plant size" (36.4%)	PC 2 "Phenology" (33.4%)	PC 3 "Growth form" (12.4%)
Flowering time	−0.1254	**0.9657**	0.0363
Reproductive period	0.1030	**−0.9229**	−0.0333
Leaves at flowering	0.3124	**0.7769**	0.0139
Diameter at flowering	**0.8378**	−0.2688	0.0486
Height	**0.7017**	0.5090	0.0148
Biomass	**0.9036**	0.1077	0.1084
Fruit number	**0.8137**	−0.1070	0.3949
Stem thickness	**0.6648**	0.5120	0.0827
Branch number	0.1928	0.0723	**0.9653**

Table 2 Traits means of *A. thaliana* plants grown at three different levels of wind intensity. The values are LS means and their standard errors extracted from the analyses of variance

Variable	Control	Low wind (~2 m/s)	Strong wind (~5 m/s)
Flowering time (d)	123.9 ± 0.3	124.4 ± 0.3	126.3 ± 0.4
Reproductive period (d)	30.1 ± 0.3	30.4 ± 0.3	29.5 ± 0.3
Leaves at flowering	34.0 ± 0.3	32.4 ± 0.3	32.1 ± 0.3
Diameter at flowering (cm)	4.81 ± 0.0	4.80 ± 0.0	4.60 ± 0.0
Height (cm)	36.5 ± 0.3	35.1 ± 0.3	31.7 ± 0.3
Biomass (mg)	93.0 ± 2.1	82.4 ± 2.1	76.8 ± 2.1
Fruit number	53.4 ± 1.1	49.2 ± 1.1	45.2 ± 1.1
Stem thickness (mm)	0.91 ± 0.0	0.88 ± 0.0	0.85 ± 0.0
Branch number	2.85 ± 0.1	2.85 ± 0.1	2.96 ± 0.1

wind treatment, when compared to the control plants, had a reduced height (–13.2%), biomass (−17.4%), and fruit number (−15.4%). The plants in the low wind treatment consistently showed an intermediate level of reduction in these traits (Table 2). While there was significant genetic variation at the family level for all of the size-related traits, and significant population or region effects for some of them (Tables 3 and 4), none of the treatment by origin interactions were significant, i.e. there was no evidence of genetic variation for plasticity. In all of the ANOVAs of size-related traits there was a significant block effect as well as a significant block by treatment interaction (Tables 3 and 4). The overall explanatory power of the full ANOVA models for these traits was moderate, leaving some 40–60% of the total variation unexplained (Tables 3 and 4).

In contrast to the size-related traits, analyses of variance of phenological traits had much smaller error components, and almost all of the variation was explained by the main effects of genetic origin (Tables 3 and 4). For example, in the case of flowering time, the full model explained almost 89% of the total variation, but 97% of this (86% of the total) was

Table 3 Analyses of variance for plant traits measured at the time of flowering

Source	DF	Flowering time		Leaves at flowering		Diameter at flowering	
		F-ratio	%Var	*F*-ratio	%Var	*F*-ratio	%Var
Block	3	**8.12*****	0.26	**25.01*****	1.65	**41.86*****	6.54
Treatment	2	**14.31*****	0.31	**12.11*****	0.53	**6.98*****	0.73
B × T	6	1.12	0.07	**7.61*****	1.00	**21.09*****	6.59
Region	3	**3.08**(*)	18.51	**3.22**(*)	18.75	**23.84*****	14.90
Population (R)	13	1.60	26.03	**2.32***	25.24	0.65	2.71
Family (P, R)	33	**115.92*****	41.37	**38.12*****	27.60	**6.19*****	10.64
R × T	6	1.17	0.24	0.29	0.05	1.63	0.27
P(R) × T	26	**1.87***	0.87	0.97	0.71	0.54	0.71
F(P, R) × T	66	**1.66*****	1.19	1.28	1.85	0.97	3.32
Error	1,031		11.15		22.62		53.61

*** Indicates effects significant at $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; (*) $P < 0.1$

Significant effects are in bold

DF = degrees of freedom

%Var = Percent of the total variance explained by a particular effect

Table 4 Analyses of variance for plant traits measured at the time of seed maturity

Source	DF	Reproduction period		Height		Biomass		Fruit number		Stem thickness		Branch number	
		F-ratio	%Var	*F*-ratio	%Var	*F*-ratio	%Var	*F*-ratio	%Var	*F*-ratio	%Var	*F*-ratio	%Var
Block	2	**6.01****	0.42	**45.88*****	4.81	**94.20*****	15.50	**89.32*****	13.11	**58.64*****	8.46	**7.08*****	1.15
Treatment	2	2.64	0.19	**50.58*****	5.30	**14.76*****	2.43	**18.12*****	2.66	**7.02*****	1.01	0.36	0.06
B × T	4	0.81	0.11	**11.39*****	2.39	**14.97*****	4.93	**16.90*****	4.96	**11.30*****	3.26	**3.30***	1.07
Region	3	1.76	10.05	0.29	1.28	0.64	0.31	**4.90***	6.51	1.11	2.84	2.33	5.91
Population (R)	13	**1.83**(*)	24.69	**2.08***	18.91	0.88	2.10	**2.89****	5.75	**1.95**(*)	11.08	**2.16***	10.97
Family (P, R)	33	**29.50*****	34.24	**13.35*****	23.07	**2.23*****	6.05	**2.09*****	5.06	**6.05*****	14.39	**4.84*****	12.92
R × T	6	1.04	0.39	1.00	0.45	0.66	0.30	0.80	0.44	1.42	0.64	**2.87***	1.38
P(R) × T	26	**1.65**(*)	1.64	1.44	1.95	0.80	1.94	1.14	2.37	1.42	1.94	1.04	2.09
F(P, R) × T	66	1.09	2.52	0.99	3.42	1.13	6.13	1.09	5.28	0.73	3.47	0.96	5.11
Error	734		25.74		38.44		60.31		53.87		52.93		59.34

*** indicates effects significant at $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; (*) $P < 0.1$

Significant effects are in bold

DF = degrees of freedom

%Var = Percent of the total variance explained by a particular effect

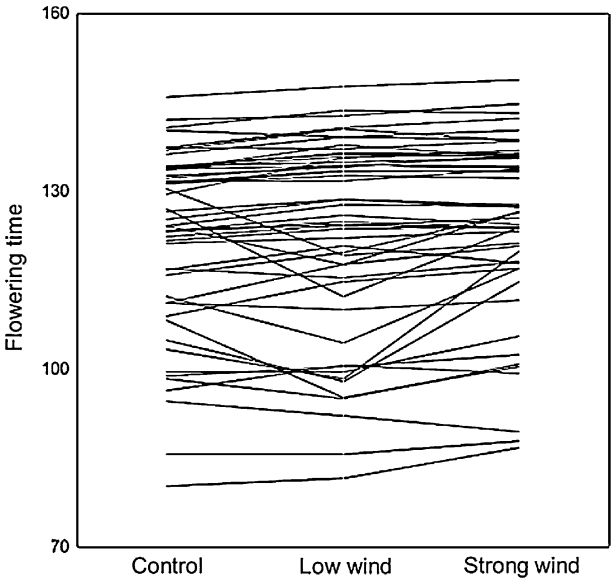

Fig. 1 The effect of mechanical stress through simulated wind on flowering time in 50 genotypes of *A. thaliana* that were collected from natural populations across Europe

due to the combined main effects of region, population and family of origin. There was a significant main effect of the wind treatments on flowering time and the number of leaves at flowering (Table 3). On average, plants in the strong wind treatment flowered 2.4 days later than the control plants, and they had significantly fewer leaves at the time of flowering (Table 2). In addition, there was significant genetic variation for plasticity in flowering time at the population and family levels (Table 3, Fig. 1), as well as a marginally significant population by treatment interaction on the length of the reproductive period (Table 4). There were also significant block effects in all analyses of phenological traits. However, compared to the main genetic effects, block and treatment effects and their

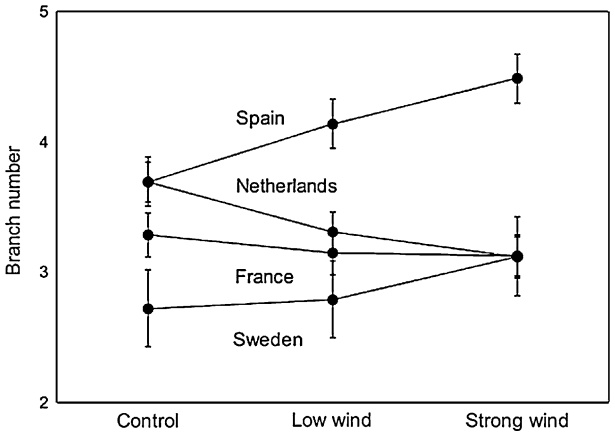

Fig. 2 The average response (± s.e.) of the total number of basal and lateral branches to mechanical stress through simulated wind in natural *A. thaliana* genotypes from four different European regions

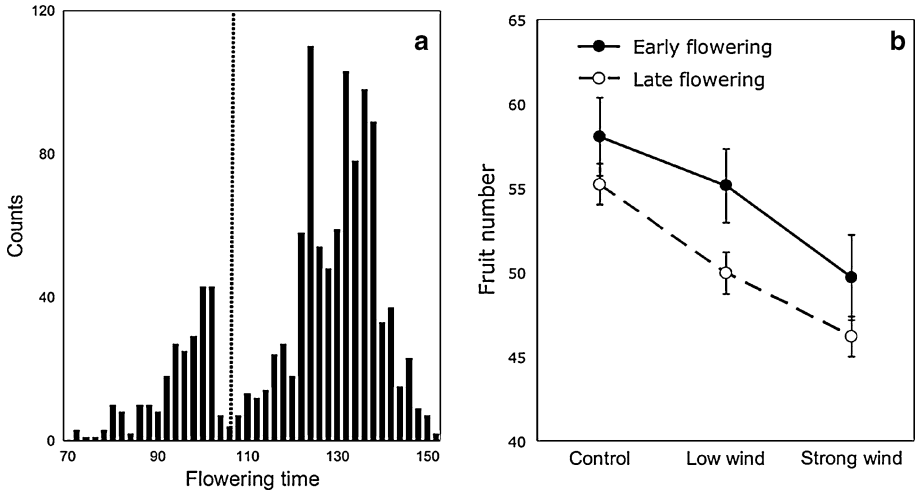

Fig. 3 (**a**) Early-flowering (left of dotted line) and late-flowering (right of dotted line) populations of *A. thaliana* that were collected from across Europe, and (**b**) their fitness response to mechanical stress through simulated wind in a controlled greenhouse experiment

interactions were not very important and together explained only a small percentage of the total variation.

There was significant genetic variation in the total number of basal and lateral branches among *Arabidopsis* populations and seed families (Table 4). In addition, while the treatment and region main effects were not significant, we found a significant treatment by region interaction with regard to branch number (Fig. 2): compared to the controls, plants from Swedish and Spanish populations that were grown in the strong wind treatment had 18% and 25% more branches, respectively, whereas in plants from French populations branch numbers remained relatively constant, and in plants from Dutch populations the average number of branches decreased by 18% (Fig. 2). There was also a significant block effect and block by treatment interaction with regard to branch number.

While the frequency distribution of flowering times across all plants was strongly bimodal (Fig. 3a), early- and late-flowering ecotypes of *A. thaliana* did not respond differently to the wind treatments (Fig. 3b). There was never a significant flowering type by treatment interaction (all $P > 0.3$) when the flowering type was included in the analyses of variance of size and growth form traits.

The correlation structure among phenotypic traits was remarkably constant across the three wind treatments. Pairwise Mantel tests between the correlation matrices for plants grown in the control, low wind and strong wind treatments (plants pooled across origins for each matrix) indicated that all matrices were highly correlated, with matrix correlations ranging between 0.965 and 0.974. A similar result was obtained using common principal components analysis, which indicated that in all three pairwise comparisons the two matrices had all principal components in common, i.e. a very similar structure of correlations among phenotypic traits. We repeated the same analysis with genetic correlation matrices (based on family means instead of individual plants in each environment) and found qualitatively very similar results, with all pairwise matrix correlations > 0.9.

There was a significant *positive* genetic correlation ($r = 0.297$, $P = 0.036$) between the cross-environment (CV) of flowering time and average plant fitness across environments (Fig. 4a). None of the other correlations between plasticity and overall fitness were

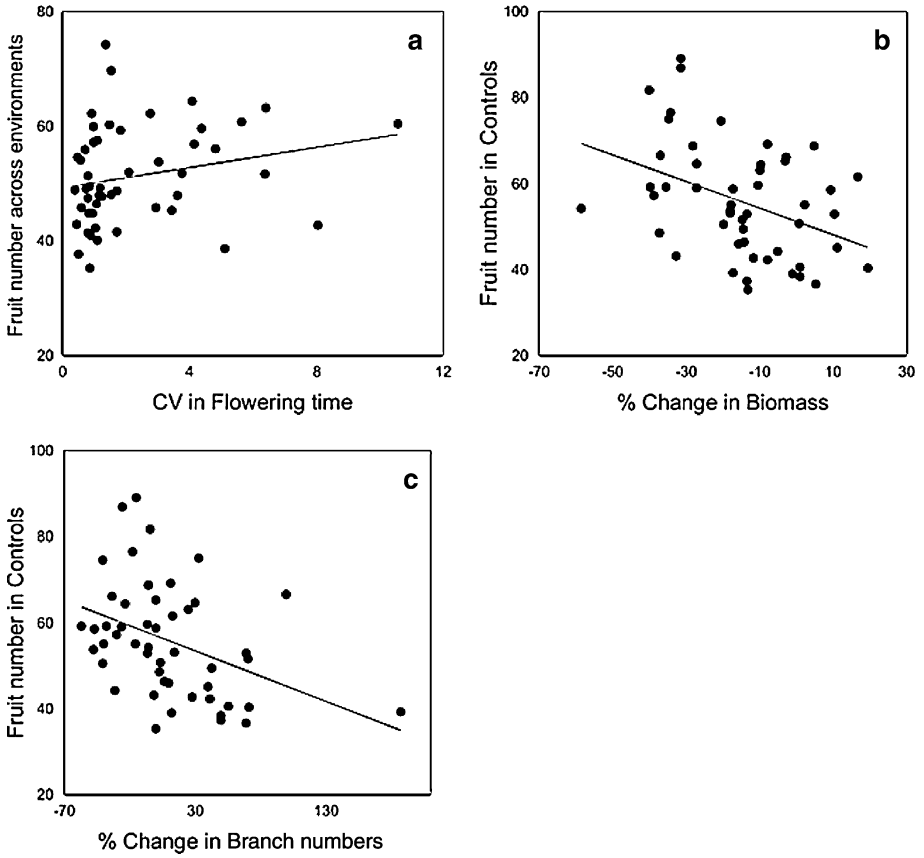

Fig. 4 Significant genetic correlations between plasticity to wind and components of plant fitness among 50 maternal families of *A. thaliana* that were collected from natural populations across Europe. Plasticity is calculated either as the coefficient of variation across three wind treatments, or as the % change in the average trait expression from the control to the strong wind treatment

significant. When we used the modified fitness averages with a different weighting of wind environments, these results remained virtually identical ($r = 0.297$–0.313 for the correlation above, depending on the weighting scheme).

There was a significant *negative* genetic correlation ($r = -0.306$, $P = 0.031$) between the CV of branch number and plant fitness in the control treatment, as well as a significant negative genetic correlation between plant fitness in the control treatment and plasticity (calculated as percentage change in the mean expression of the trait from the control to the strong wind treatment) with regard to diameter at flowering ($r = -0.325$, $P = 0.021$), plant biomass ($r = -0.418$, $P = 0.003$; Fig. 4b), and branch number ($r = -0.343$, $P = 0.015$; Fig. 4c).

Discussion

Thigmomorphogenesis refers to the phenotypic changes by which plants respond to mechanical stimulation. However, in contrast to responses to other environmental factors

such as light, nutrients or water, we still know little about the genetics and evolution of this type of phenotypic plasticity. Here, we studied genetic variation for thigmomorphogenesis in response to wind within and among natural populations of *A. thaliana*.

Our experiment was conducted in an unheated greenhouse with ambient light. This provided realistic growth conditions; the plants were naturally vernalized and they experienced normal fluctuations of light and temperature. The disadvantage of this set-up was that environmental conditions were more heterogeneous in the greenhouse bay than they would have been in a climate-controlled greenhouse, which is reflected in the large block effects in all analyses of variance. However, we think that the blocking successfully captured much of the heterogeneity in our experiment, and that the advantages of our relatively uncontrolled set-up outweigh this disadvantage.

Overall response to wind

Mechanical stimulation through wind significantly delayed the flowering of plants, and it reduced their overall growth and reproduction. While the general direction of these results is in accordance with previous studies (e.g., Whitehead 1962; Henry and Thomas 2002; Anten et al. 2005), the effect of wind was surprisingly small. Even strong wind had relatively little effect on plant phenotypes, and, consequently, the wind treatments never explained more than a few percent of the total phenotypic variation. One conclusion from our experiment is that *A. thaliana* appears to be rather tolerant to the mechanical stress imposed by wind.

Obviously, an alternative explanation would be that our wind treatments were not strong enough. However, the visual impression of our experiment does not support this. In the strong wind treatment, the plants were strongly bent and shaken, and we do not think it would have been possible to increase the wind speed even further without causing significant damage through uprooting or stem rupture. The wind speeds used in our experiment were chosen on the basis of actual wind statistics, and compared to previous studies (e.g. Whitehead 1962; Cipollini 1997, 1998; Henry and Thomas 2002; Pigliucci 2002; Murren and Pigliucci 2005) they were average. Whitehead (1962) used a range of wind speeds from 1–15 m/s and found strong phenotypic effects already at 4 m/s. We therefore think it reasonable to assume that our wind treatments were strong enough to potentially trigger a thigmomorphogenetic response.

We found no evidence for some of the architectural changes that are often mentioned as common aspects of thigmomorphogenesis in plants (Biddington 1986; Braam et al. 2005). Stem thickness did not increase in response to wind but co-varied allometrically with plant size and thus decreased in stimulated plants. We also found no general increase in branch number. Instead, the effect of wind on branching depended on plant origin. While some of these inconsistencies with the allegedly general plasticity syndrome in response to mechanical stress may be due to specific developmental constraints in *A. thaliana*, we think that the answer may lie in the common, but probably incorrect, assumption that from a plant's point of view wind and actual physical touch are the same.

For instance, Cipollini (1998) showed that, while wind and brushing elicited the same kind of biochemical response in common bean, the plants appeared to be generally more sensitive to brushing. Another very informative study in this context was carried out by Smith and Ennos (2003), who showed that while flexing reduced the height of sunflowers and increased the strength of their stems—which is in line with the common description of thigmomorphogenesis—air flow had in fact the opposite effect: it increased plant height

and decreased stem rigidity. Wind treatments as in our study have a flexing and an air flow component, therefore experiments that use wind as mechanical stimulus should not be expected to give the same results as those that use bending, brushing, or similar approaches. Interestingly, most previous studies that found increased stem diameter subjected plants to flexure (e.g., Cipollini 1999; Anten et al. 2005), whereas most studies that used wind found no or even the opposite effect (Henry and Thomas 2002; Smith and Ennos 2003; this study). Altogether, there appears to be sufficient evidence to conclude that, while there might be some commonalities in plant responses to different kinds of mechanical stress, there is no universal thigmomorphogenetic response. Hence, the results of our study should only be interpreted with regard to the effects of wind—which is probably the most common form of mechanical stress in nature.

Phenotypic correlations

Typically, phenotypic traits are not independent from each other, but there are patterns of trait covariation that reflect genetic, functional, or developmental relationships and affect the magnitude and direction of evolutionary responses (Schlichting 1989; Arnold 1992; Pigliucci and Preston 2004). Here, we found that phenotypic traits of *A. thaliana* were organized into three distinct groups of correlated traits—or phenotypic modules (Wagner and Altenberg 1996)—that represented variation in plant size, phenology, and growth form. This modular correlation structure was extremely stable across environments. Thus, we found no evidence for thigmomorphogenesis to be a multivariate "plasticity syndrome" (Smith and Whitelam 1997; Pigliucci 2002) with a characteristic shift in the phenotypic correlation structure. This result was fairly robust and did not change when we used a different method of matrix comparison, or when we examined genetic instead of phenotypic correlation matrices.

There are two major hypotheses typically advanced to explain stable phenotypic correlations as the ones observed here (Armbruster and Schwaegerle 1996; Merilä and Björklund 2004): First, phenotypic correlations may be the "inevitable" result of shared genetic control (pleiotropy or linkage) of different traits. Second, since phenotypes evolve as wholes, and phenotypic integration is likely to ensure coherence between different parts of a phenotype, phenotypic correlations may have evolved by natural selection. More specifically, a modular correlation structure, with strong correlations within but weak correlations among modules, may be advantageous because it allows quasi-independence of different parts of an organism (Wagner and Altenberg 1996). While the question of phenotypic integration as a constraint versus adaptation cannot be answered with our data, our findings have two important evolutionary implications: in our study system (1) traits within the same module are bound to evolve together, at least in the short term, and (2) there is a potential for the different modules to evolve independently, i.e. the response of phenological traits to environmental challenges such as mechanical stimulation will be largely independent of growth and size-related traits.

Potential for evolutionary change

The basic requirements for phenotypic evolution are: (1) heritable variation and (2) selection acting on this variation. Here, we were interested in the potential for evolution of thigmomorphogenetic plasticity. We used the wind-by-origin interactions in the ANOVAs as a test of genetic variation for plasticity to wind, and we estimated selection on plasticity by calculating family-level correlations between the average degree of plasticity of a trait and the

average fitness of a family across environments (Scheiner 1993; Via et al. 1995). In addition, we estimated costs of plasticity (DeWitt et al. 1998) by calculating family-level correlations between plasticity estimates and the average fitness of plants in the control treatment.

There appears to be little potential for evolutionary change in the thigmomorphogenetic response of size-related traits in *A. thaliana*. While these traits were generally highly plastic and genetically variable, we found no evidence of genetic variation for plasticity, i.e. none of the wind by origin interactions in ANOVAs of size-related traits were significant. Evolution of plasticity in this phenotypic module may thus be constrained by a lack of raw material for selection to act upon. One explanation for this could be that strong past selection has depleted genetic variation. Phenotypic traits closely related to fitness often have lower levels of additive genetic variation than traits less closely related to fitness (Mousseau and Roff 1987; Price and Schluter 1991). Another piece of evidence comes from plasticity-fitness correlations, which show that the better a family is able to maintain its biomass under strong wind, the lower is its fitness in the absence of wind. Tolerance to wind may thus incur a fitness cost, and strong selection for high levels of plasticity is therefore unlikely. It is conceivable that the observed intermediate level of plasticity in size-related traits is a result of some form of balancing selection created through a combination of antagonistic processes.

The situation is different when we look at the module of phenological traits. Here, we found a great amount of genetic variation among plants of different origins—which is typical in *A. thaliana* (e.g., Stinchcombe et al. 2004; Lempe et al. 2005)—but the plasticity of these traits was rather limited. There was significant genetic variation for plasticity of flowering time, which indicates a potential for plasticity in this trait (and module) to evolve. Moreover, there was a significant positive relationship between the CV of flowering time and the average fitness of families across environments: families that showed greater plasticity in flowering time had a higher fitness across environments. This correlation was very robust; it did not change when we assumed different frequencies of environments in our calculation of fitness averages. Assuming our experimental set-up successfully mimicked, at least to some extent, the environmental conditions in natural populations of *A. thaliana*, the positive plasticity-fitness correlation indicates directional selection for increased phenological plasticity (delayed flowering) in response to wind, and therefore it suggests that this aspect of thigmomorphogenesis is adaptive.

Variation in total branch number, a trait that describes plant architecture in *A. thaliana*, was largely independent from other phenotypic traits. While there was significant regional variation in the degree of plasticity of this trait—wind strongly increased branch numbers in genotypes from Spanish populations, but not in those from other European regions—we found no evidence of genetic variation for plasticity among families. Moreover, plasticity in branch numbers appeared to have a fitness cost: the greater the average increase of branch number in response to wind, the lower was the average fitness of a family in the absence of wind. Together, this suggest that—as in the plant size module—evolution of plasticity may be constrained by a lack of genetic variation within populations, costs of plasticity, or a combination of both. The observed regional variation in plasticity could be the result of regional variation in antagonistic selection factors. It is conceivable, for instance, that in the drier Spanish populations a branched growth form confers a fitness advantage, because it reduces water losses through transpiration. This advantage may counterbalance the intrinsic fitness costs of increased branching, so that natural selection favours increased plasticity of branch numbers in this environment, while it may do the opposite in other regions.

We have used plasticity-fitness correlations as a test of the adaptive significance of plasticity to wind. Admittedly, there is an inherent difficulty in the study of adaptive plasticity through selection analyses in the greenhouse—even though this has been a common approach (Scheiner 1993; Via et al. 1995)—as one may always question the ecological relevance of controlled environments. Another approach, for instance, would be to use phenotypic manipulation (Schmitt et al. 1999) for studying the performance of differently induced phenotypes when transplanted into contrasting natural habitats. Still, testing for adaptive plasticity is an inherently difficult problem (Sultan 2000; Pigliucci 2005), and every approach has its advantages and disadvantages. Ideally, a thorough analysis of adaptive plasticity will combine several lines of evidence, and our selection analyses should be regarded as a first step towards understanding the adaptive significance of thigmomorphogenesis.

Conclusions

While thigmomorphogenesis, the phenotypic changes associated with mechanical stimulation in plants, is a well-known phenomenon, we still know little about the genetics and evolution of this type of phenotypic plasticity in natural populations. Here, we examined genetic variation for thigmomorphogenesis in natural populations of *A. thaliana*. We found that overall the plants were remarkably tolerant to the mechanical stress inflicted by wind. It is possible that this reflects adaptation of *A. thaliana* to its open habitats. Nevertheless, wind did have a significant effect on plant phenology, growth and reproduction, and we found that there was natural genetic variation for this response and therefore a potential for it to evolve. Finally, phenotypic traits were organized into distinct modules that showed different responses to wind, and may therefore to some extent evolve independently from each other.

To elucidate the genetic basis and evolutionary implications of these findings, future research should combine existing knowledge about the physiological and molecular underpinnings of thigmomorphogenesis (Braam 2005) with phenotypic data obtained from ecological experiments like the one described here. This should yield a more mechanistic understanding of the observed phenotypic changes, and at the same time it would provide a test of the ecological relevance of proposed key physiological processes and candidate genes. Another avenue of future research should be the study of thigmomorphogenesis in a comparative framework, i.e. in groups of related species (Murren and Pigliucci 2005), to address macroevolutionary questions such as when and how often the thigmomorphogenetic response has evolved in plants.

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References

Al-Shehbaz IA, O'Kane SL (2002) Taxonomy and phylogeny of *Arabidopsis* (Brassicaceae). In: Somerville CR, Meyerowitz EM (eds) The arabidopsis book, Am Soc Plant Biol, Rockville, doi: 10.1199/tab.0001, http://www.aspb.org/publications/arabidopsis/

Anten NPR, Casado-Garcia R, Nagashima H (2005) Effects of mechanical stress and plant density on mechanical characteristics, growth, and lifetime reproduction of tobacco plants. Am Nat 166:650–660

Armbruster WS, Schwaegerle KE (1996) Causes of covariation of phenotypic traits among populations. J Evol Biol 9:261–276
Arnold SJ (1992) Constraints on phenotypic evolution. Am Nat 140:S85–S107
Bergelson J, Stahl E, Dudek S et al (1998) Genetic variation within and among populations of *Arabidopsis thaliana*. Genetics 148:1311–1323
Biddington NL (1986) The effects of mechanically-induced stress in plants—a review. Plant Growth Regul 4:103–123
Biddington NL, Dearman AS (1985) The effect of mechanically induced stress on the growth of cauliflower, lettuce and celery seedlings. Ann Bot 55:109–119
Braam J (2005) In touch: plant responses to mechanical stimuli. New Phytol 165:373–389
Braam J, Davis RW (1990) Rain-, wind- and touch-induced expression of calmodulin and calmodulin-related genes in *Arabidopsis*. Cell 60:357–364
Braam J, Sistrunk ML, Polisensky DH et al (1996) Life in a changing world: *TCH* gene regulation of expression and responses to environmental signals. Physiol Plantarum 98:909–919
Cahill JF, Castelli JP, Casper BB (2001) The herbivory uncertainty principle: visiting plants can alter herbivory. Ecology 82:307–312
Callahan HS, Pigliucci M (2002) Shade-induced plasticity and its ecological significance in wild populations of *Arabidopsis thaliana*. Ecology 83:1965–1980
Cipollini DF (1997) Wind-induced mechanical stimulation increases pest resistance in common bean. Oecologia 111:84–90
Cipollini DF (1998) The induction of soluble peroxidase activity in leaves of bean plants by wind-induced mechanical perturbation. Am J Bot 85:1586–1591
Cipollini DF (1999) Costs to flowering of the production of a mechanically hardened phenotype in *Brassica napus* L. Int J Plant Sci 160:735–741
DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends Ecol Evol 13:77–81
Ennos AR (1997) Wind as an ecological factor. Trends Ecol Evol 12:108–111
Garner LC, Björkman T (1996) Mechanical conditioning for controlling excessive elongation in tomato transplants: sensitivity to dose, frequency, and timing of brushing. J Am Soc Hortic Sci 121:894–900
Henry HAL, Thomas SC (2002) Interactive effects of lateral shade and wind on stem allometry, biomass allocation, and mechanical stability in Abutilon theophrasti (Malvaceae). Am J Bot 89:1609–1615
Jaffe MJ (1973) Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation. Planta 114:143–157
Jaffe MJ, Leopold AC, Staples RC (2002) Thigmo responses in plants and fungi. Am J Bot 89:375–382
Johnson KA, Sistrunk ML, Polisensky DH et al (1998) *Arabidopsis thaliana* responses to mechanical stimulation do not require ETR1 or EIN2. Plant Physiol 116:643–649
Jørgensen S, Mauricio R (2004) Neutral genetic variation among wild North American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. Mol Ecol 13:3403–3413
Kern KA, Ewers FW, Telewski FW et al (2005) Mechanical perturbation affects conductivity, mechanical properties and aboveground biomass of hybrid poplars. Tree Physiol 25:1243–1251
Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu Rev Plant Biol 55:141–172
Latimer JG (1998) Mechnical conditioning to control height. Hort Technol 8:529–534
Lempe J, Balasubramanian S, Sureshkumar S et al (2005) Diversity of flowering responses in wild *Arabidopsis thaliana* strains. PLoS Genetics 1:109–118
Lundqvist L, Valinger E (1996) Stem diameter growth of Scots pine trees after increased mechanical load in the crown during dormancy and (or) growth. Ann Bot 77:59–62
Malakoff D (2004) Measuring the significance of a scientist's touch. Science 306:801
Meinke DW, Cherry JM, Dean C et al (1998) *Arabidopsis thaliana*: a model plant for genome analysis. Science 282:662–682
Merilä J, Björklund M (2004) Phenotypic integration as a constraint and adaptation. In: Pigliucci M, Preston K (eds) Phenotypic integration: Studying the ecology and evolution of complex phenotypes. Oxford University Press, Oxford, pp 107–129
Mitchell-Olds T (2001) *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. Trends Ecol Evol 16:693–700
Mousseau TA, Roff DA (1987) Natural selection and heritability of fitness components. Heredity 59:181–197
Murren CJ, Pigliucci M (2005) Morphological responses to simulated wind in the genus *Brassica (Brassicaceae)*: allopolyploids and their parental species. Am J Bot 92:810–818
Newman JA, Bergelson J, Grafen A (1997) Blocking factors and hypothesis testing in ecology: Is your statistics text wrong? Ecology 78:1312–1320

Nordborg M, Hu TT, Ishino Y et al (2005) The pattern of polymorphism in *Arabidopsis thaliana*. PLoS Biology 3:1289–1299
Oke TR (1987) Boundary layer climates. Methuen, London
Pang PP, Meyerowitz EM (1987) *Arabidopsis thaliana*: a model system for plant molecular biology. Bio/Technol 5:1177–1181
Pigliucci M (2002) Touchy and bushy: phenotypic plasticity and integration in response to wind stimulation in *Arabidopsis thaliana*. Int J Plant Sci 163:399–408
Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol 20: 481–486
Pigliucci M, Preston K (eds) (2004) Phenotypic integration: Studying the ecology and evolution of complex phenotypes. Oxford University Press, Oxford
Pigliucci M, Cammell K, Schmitt J (1999) Evolution of phenotypic plasticity: a comparative approach in the phylogenetic neighbourhood of *Arabidopsis thaliana*. J Evol Biol 12:779–791
Price T, Schluter D (1991) On the low heritability of life history traits. Evolution 45:853–861
Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. Annu Rev Ecol Syst 24:35–68
Schlichting CD (1989) Phenotypic integration and environmental change. Bioscience 39:460–464
Schmitt J, Dudley SA, Pigliucci M (1999) Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. Am Nat 154:S43–S54
Sharbel TF, Haubold B, Mitchell-Olds T (2000) Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. Mol Ecol 9:2109–2118
Sistrunk ML, Antosiewicz DM, Purugganan MM et al (1994) Arabidopsis TCH3 encodes a novel Ca2 + binding protein and shows environmentally induced and tissue-specific regulation. Plant Cell 6:1553–1565
Smith VC, Ennos AR (2003) The effects of air flow and stem flexure on the mechanical and hydraulic properties of the stems of sunflowers *Helianthus annuus* L. J Exp Bot 54:845–849
Smith H, Whitelam GC (1997) The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant Cell Environ 20:840–844
Stenøien HK, Fenster CB, Tonteri A et al (2005) Genetic variability in natural populations of *Arabidopsis thaliana* in northern Europe. Mol Ecol 14:137–148
Steppan SJ, Phillips PC, Houle D (2002) Comparative quantitative genetics: evolution of the G matrix. Trends Ecol Evol 17:320–327
Stinchcombe JR, Weinig C, Ungerer M et al (2004) A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene FRIGIDA. Proc Natl Acad Sci USA 101:4712–4717
Sultan S (2000) Phenotypic plasticity for plant development, function and life history. Trends Plant Sci 5:537–542
Tonsor SJ, Alonso-Blanco C, Koornneef M (2005) Gene function beyond the single trait: natural variation, gene effects, and evolutionary ecology in *Arabidopsis thaliana*. Plant Cell Environ 28:2–20
Troen I, Petersen EL (1989) European wind atlas. Risø National Laboratory, Roskilde
Via S, Gomulkiewicz R, De Jong G et al (1995) Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol Evol 10:212–217
Wagner GP, Altenberg L (1996) Complex adaptations and the evolution of evolvability. Evolution 50:967–976
Whitehead FH (1962) Experimental studies of the effect of wind on plant growth and anatomy. II. *Helianthus annuus*. New Phytol 61:59–62
Xu W, Purugganan MM, Polisensky DH et al (1995) Arabidopsis TCH4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. Plant Cell 7:1555–1567